# OOGENESIS AND FERTILIZATION IN *THERMOBIA DOMESTICA* (PACKARD)[1]

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The materials and methods used in this study were the same as those described in an earlier paper (Woodland, 1957).

## *Oogenesis*

The primary oocytes in the vitellarium reach a length of about 420 microns just before yolk accumulation begins. They measure only about 45 microns wide. Each has a large central nucleus about 40 microns in diameter. It is a typical vesicular oocyte nucleus and usually has a visible nuclear membrane. A fine network is visible throughout the nucleus and there is a large, eccentric, irregular, granular nucleolus which may be as long as 20 microns. Neither network nor nucleolus is Feulgen-positive. The finely-divided chromatin is so scattered throughout the large nucleus that it is barely visible. The cytoplasm appears very finely granular and contains a few inclusions. These are 10 to 15 microns in diameter and consist of a dozen or less clumped globules.

When each of the vitellaria contains four or five of these oocytes, yolk accumulation begins. It starts peripherally in all of the oocytes simultaneously and gradually proceeds toward the center. There appear scattered through the peripheral cytoplasm tiny globules which stain bright orange-red with Mallory's triple stain. They greatly resemble the globules of protein reserves scattered through the fat body surrounding the ovarioles, but are usually a little smaller. The negative images of small fat droplets also occur in the peripheral cytoplasm. The fat droplets and proteinaceous globules increase in size, forcing the diminishing cytoplasm into a network around them. By

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the time that small fat droplets begin to appear in the center of the oocyte, the peripheral droplets and proteinaceous globules have greatly enlarged (3 to 7 microns in diameter) and are present in about equal numbers. The diminishing central layer of cytoplasm around the nucleus is connected by the fine network between the yolk globules to a thin peripheral cytoplasmic layer, the periplasm. By the time the perinuclear cytoplasm has become very thin, the fat droplets appear to have coalesced, for the visible proteinaceous yolk globules, some of which are now as large as 11 microns in diameter, stand out in fixed preparations against a clear background.

The oocyte has been growing only slightly meanwhile, and is still very narrow in proportion to its length. Before oviposition the maximum length of the egg increases by about two and one-half times, and its maximum width increases about eighteen times. Growth is accomplished chiefly by an increase in the proteinaceous yolk. Just before oviposition the egg contains relatively little fatty yolk.

At the start of the growth period the nucleus moves to the periphery. The nuclear network condenses at the periphery of the nucleus and the chromatin becomes distinctly visible with the Feulgen technique. Evidence of tetrads is seen. The interior of the large nucleus now appears homogeneous and has little affinity for the stains used. Before oviposition there occur both a marked decrease in nuclear volume and further condensation of the chromatin into small compact chromosomes.

The follicle cells start to secrete the endochorion at the time when yolk accumulation first begins. The process is best studied in preparations stained with phosphotungstic hematoxylin. Just before secretion of the endochorion starts, the brown-staining connective tissue sheath surrounding the ovariole becomes greatly thickened. It appears that the follicle cells withdraw material from the sheath and secrete it to form the endochorion. Comparison with the accompanying process of yolk accumulation indicates that the sheath is reduced to its former size within a relatively short time. The secretion of the exochorion, which occurs much later, is mentioned below.

The external appearance of the newly laid egg of the firebrat has been described by Adams (1933), Sweetman (1938), and Remington (1948). The present study reveals that the endochorion (Fig. 5, EN) is about 2 microns thick. Its thickness does not vary appreciably over the whole egg. There adheres to its outer surface a single layer of spherical droplets, each about 11 microns in diameter immediately after oviposition. The droplets are rather irregularly placed, but tend to be in groups with large intervening spaces. In the spaces are droplets many times smaller than the large ones. Within a few hours a change occurs. The large droplets break up into rather evenly spaced ones about 3 microns in diameter (Fig. 5, CD). Usually a space of 2 microns now occurs between droplets, although pairs of contiguous droplets are sometimes seen. The very minute droplets are still scattered among the larger ones. The appearance of the droplets is essentially the same on shed chorions long after the nymphs have left them. Possibly the droplets are present as a thin continuous film before oviposition.

The droplets adhere firmly to the exochorion. A few individual droplets are separated from it by sectioning, but the other procedures to which the eggs have been subjected practically never dislodge them. In contrast, the exochorion is loosened from the endochorion when the egg contents shrink during fixation, owing to dissolution of the fatty elements. Moreover, the exochorion is normally loosened from the endochorion during development, as the volume of the egg contents is diminished slightly. The surface of the endochorion is then seen to be reticulated into hundreds of small polygonal areas. Most of these polygons are fairly regular hexagons with diagonals usually between 55 and 85 microns long. The wall separating adjacent areas is 5 microns thick.

Sweetman (1938) supposed this hexagonal reticulation to be produced by the cells of the embryonic tissues, but the present study showed it to be produced by the follicular cells that secreted the endochorion. The large size of the hexagons emphasizes the amount the egg has grown since the endochorion was secreted.

The endochorion is hardly ever loosened at all from the yolk and no vitelline membrane has been found between them. Heymons (1897) reported a vitelline membrane in *Lepisma saccharina,* but Uzel (1898), who studied the eggs of the same lepismatid, did not mention it.

Between the exochorion and the endochorion at the anterior end of the egg is the micropylar area, a circular thickening about 280 microns in diameter (Fig. 5). The thickening consists of as many as twenty or more concentric lamellae, each about as thick as the exochorion, with which the thickening is identical in staining reactions and to which it adheres if the latter becomes loosened from the endochorion. The thickening is thinner peripherally than centrally, since not all of the lamellae extend to the margin of the area.

In the micropylar area are found three small infundibular depressions which, if connected, would form the corners of an equilateral triangle. Two of the depressions are shown in figure 5 (DP). The depressions, which can rarely be demonstrated to penetrate the micropylar thickening completely, are filled with folds of the exochorion and with the chorionic droplets described above. There sometimes ap-

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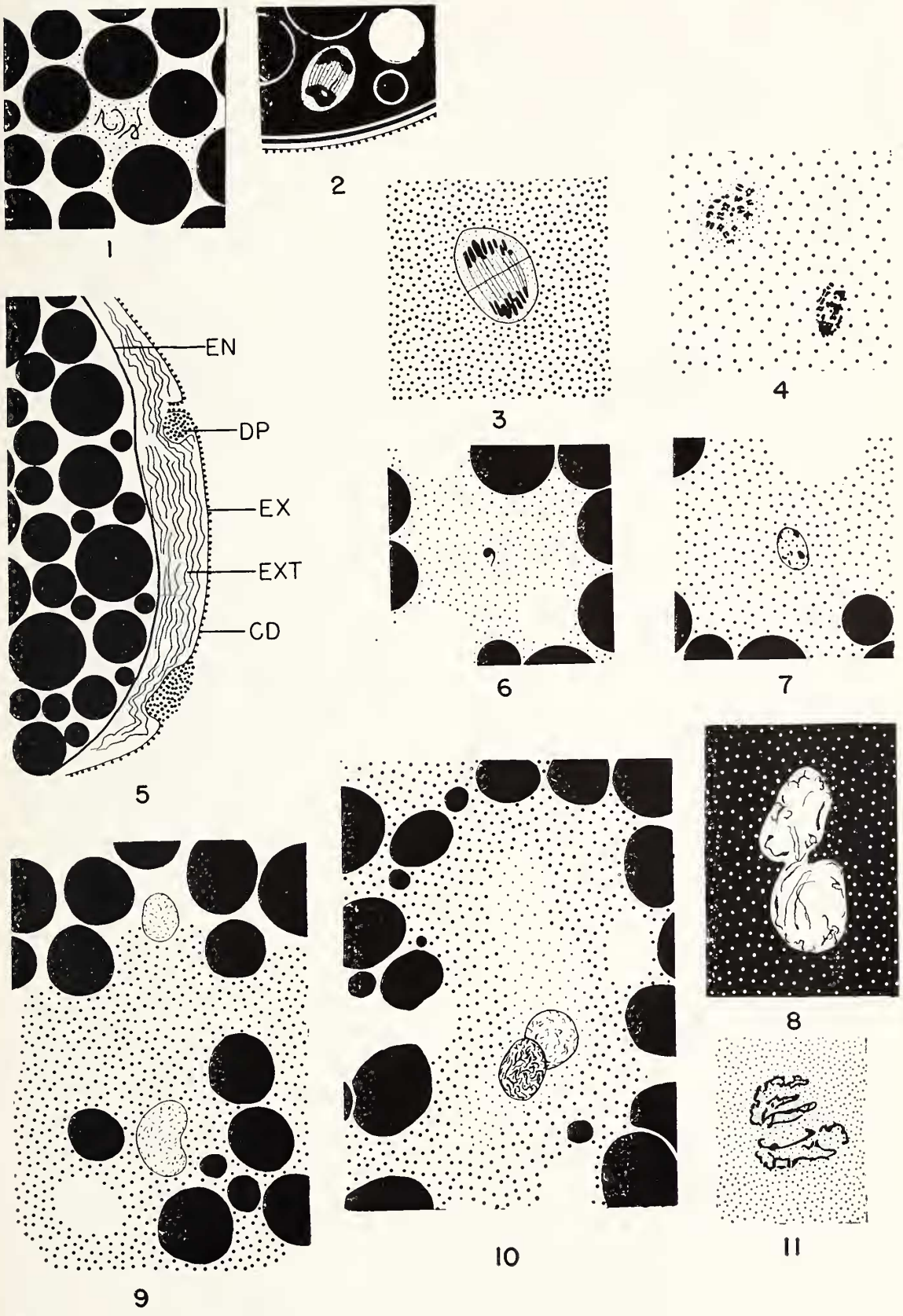
Explanation of Plate 2

All figures represent portions of sections. Fixative: Maximow's. Stain: Feulgen's all figs. except 2 and 5; Mallory's triple, figs. 2 and 5. Magnifications approximate. Fig. 1. 20 minute old egg, showing sperm head accumulating cytoplasm, 400 x. Fig. 2. 30 minute old egg, showing egg nucleus in anaphase of first maturation division, 500 x. Fig. 3. 2½ hour old egg, showing egg nucleus in anaphase of second maturation division, 500 x. Fig. 4. 75 minute old egg, showing late telophase of first maturation division; secondary oocyte nucleus forming in upper left, first polar body in lower right; 500 x. Fig. 5. Longitudinal section through micropylar (anterior) end of 2 hour old egg, 160 x; CD, chorionic droplet; DP, depression; EN, endochorion; EX, exochorion; EXT, exochorionic thickening. Fig. 6. 90 minute old egg, showing late stage in the contraction of the sperm head, 1250 x. Fig. 7. Egg about 2 hours old, showing male pronucleus during its growth period; 1875 x. Figs. 8-11. 3 to 4 hour old eggs, 1250 x. Fig. 8. Early stage of union of male and female pronuclei. Fig. 9. Female pronucleus shown near top just enteriing the sperm plasm; male pronucleus (below). Fig. 10. Female pronucleus (below) and male pronucleus (above) about to unite. Fig. 11. Late stage of union of male and female pronuclei.

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pears to be an opening through the exochorion where it dips into a depression (Fig. 5), but no opening through the endochorion was found. Other evidence suggests that there is none. Eggs completely immersed in tap water at 37.5° C. for a period of 3 or 4 days during incubation hatch into healthy nymphs in about the normal time. (Hatching itself occurs under water, but the nymphs cannot get their abdomens out of the shell. This is because they cannot get traction and also occurs in a small percentage of eggs hatching in an empty glass container.) If openings completely penetrated the chorion, then water should be expected to enter the egg by osmotic pressure and cause it to burst. If eggs are kept in tap water for as long as seven days, the incubation period is prolonged by several days and only about half of the eggs hatch at all. Those not hatching do not burst nor are they turgid. Presumably they die from oxygen lack, as the water was not mechanically oxygenated.

The follicle cells appear to become a syncytium during the enlargement of the oocyte. This syncytium probably secretes the micropylar thickening and the thin structureless exochorion just before the oocyte leaves the ovariole. The chorionic droplets are believed to be added as the egg passes through the lateral oviduct, for droplets of similar appearance have been seen in the cells of the oviducal epithelium of females with large oocytes in the ovarioles.

### *Fertilization*

After the oocyte leaves the gonopore but before it proceeds down the ovipositor, it is undoubtedly held at the base of the ovipositor for a few moments while sperms are discharged onto it by the spermatheca. The sperms presumably enter the probable openings in the exochorion over the depressions in the micropylar thickening. They can apparently penetrate the endochorion directly. They would not have to penetrate the endochorion directly under the micropylar thickening, but could pass between the lamellae of the thickening and enter nearer the equator of the egg. Whether the entire sperm penetrates the egg was not determined. Within the freshly laid egg, however, may usually be identified from one to six of the very long sperm heads,

each of which is bent and coiled upon itself many times.

The contents of the living, freshly laid egg appear to consist of colorless yolk spheres in a colorless liquid of low viscosity. The spheres are about 10 to 15 microns in diameter. In sections of eggs fixed with Maximow's fluid the negative images of the fat spheres measure 9 or 10 microns in diameter. Most of the more numerous, visible, proteinaceous yolk globules are 14 or 15 microns in diameter. Some of these globules appear structureless, while others appear finely granular. The globules appear only very slightly flattened at the poles with Maximow's fixative. The other fixatives used always badly distorted the yolk of young eggs. The yolk spheres and liquid fill the entire egg. Cytoplasm was not identified.

Perrot (1933) stated that the first maturation division occurs while the oocyte is still in the ovariole. He was not able to find any trace of the prophase of the division. He reported that the mitotic figure occupies a very small space at the periphery of the oocyte and figured the anaphase of the division. He stated further that after this first maturation division the nucleus enters a resting stage which he figured from a freshly laid egg. The present study did not confirm this part of Perrot's work. The only mitotic figures ever found in sections through the vitellarium were those of the follicle cells. The anaphase of such follicular mitoses often resembles that shown in the first of Perrot's figures just mentioned. Eggs fixed immediately after oviposition show the nucleus to be in the metaphase of the first maturation division. No nucleus resembling both in appearance and position that shown in the second of Perrot's figures was found until the beginning of the formation of the primary epithelium.

The anastral type of meiosis occurs. The mitotic figure is located more or less equidistant from the two poles of the egg, usually from 1 to 10 microns from the surface. The spindle axis is usually oblique to the egg surface, but is occasionally parallel to it. The distance between the two poles of the mitotic figure in metaphase and in anaphase (Fig. 2) is about 20 microns. The metaphase plate is about 8 microns in diameter. The chromosomes are very small

and close together. During telophase the mitotic figure is no longer fusiform, but is a spheroid with long axis about 14 microns long and short axis about 10 microns long. In presumably slightly older eggs a very distinct equatorial constriction is evident. The two groups of chromosomes separate to a distance of almost 30 microns. In favorably sectioned eggs most of the eighteen dyads in each group can be distinguished (Fig. 4). Many of the chromosomes are very short and V-shaped, so that the dyads look superficially like tetrads.

The first polar body is not extruded from the egg and does not divide. Its chromosomes never become surrounded by a nuclear membrane as do those of the secondary oocyte. The dyads gradually change into an irregular mass, which remains close to the surface of the egg and disappears relatively quickly. No certain trace of the first polar body was found later than the anaphase of the second maturation division.

The egg nucleus was never found in a resting stage between the maturation divisions. The spheroidal prophase nucleus, with its still distinguishable dyads, is believed to increase in size, since a series has been found with long axis varying from 8 to 15 microns in length. The second maturation division resembles the first in size and orientation, but its chromosomes are slightly more easily distinguishable (Fig. 3). The second polar body degenerates as does the first and has not been seen with certainty later than the four-celled cleavage stage. The chromosomes of the female pronucleus enter a resting stage with distinct nuclear membrane. The nucleus is a spheroid with long and short diameters of 9 microns and 5 microns respectively. It shows hardly any variation from egg to egg.

In the majority of freshly laid eggs, cytoplasm is seen starting to accumulate around one of the sperm heads (Fig. 1). This sperm and its sperm plasm are typically found on the longitudinal axis of the egg, about one-third of the way from the posterior pole to the anterior pole. The distance from the maturation spindle is roughly 400 microns. If several sperms are present in the egg, cytoplasm may or may not accumulate around more than one. In cases where

cytoplasm accumulates around from two to six sperms, their distribution is such that they are about equidistant from each other and from the surface of the egg. Just as much cytoplasm accumulates around a sperm whether it is the only one accumulating cytoplasm or whether five others are doing likewise. Accumulation of the sperm plasm ceases at about the time the oocyte nucleus has completed its first maturation division. By that time the sphere of sperm plasm is about 45 microns in diameter.

As cytoplasm accumulates around a sperm head, the latter undergoes a gradual decrease in length, with accompanying uncoiling and moderate increase in breadth. The Feulgen reaction indicates a simultaneous concentration of desoxyribonucleic acid toward the anterior end of the shortening sperm head (Fig. 6). Shortening continues until all that is visible within the sperm plasm is a minute nucleus about 1½ microns long. This male pronucleus is formed at about the time that accumulation of sperm plasm ceases. While the egg nucleus is undergoing the second maturation division, the male pronucleus gradually enlarges (Fig. 7) until it is about 8 or 10 microns in diameter (Fig. 9).

The female pronucleus then migrates from its peripheral position to the sperm plasm (Fig. 9). When fixed before it reaches the sperm plasm, it is found to be rather irregularly elongated in the direction in which it was presumably travelling. Cytoplasm has never been identified around it. Both male and female pronuclei are approximately the same size and their finely divided chromatin appears very similar. Fortunately they can easily be distinguished when the Feulgen reaction is followed by light green counterstain. The nuclear sap of the male pronucleus shows great affinity for light green, while that of the female pronucleus shows practically none at all. It appears further that the movement of each nucleus within the sperm plasm just prior to fixation can be detected. For adjoining each nucleus fixed at this stage is a palely-staining streak which is interpreted to indicate the path over which the nucleus has just passed (Fig. 10).

As the female pronucleus enters the sperm plasm the sperm nucleus starts to move toward it. The two pronuclei

come together and their nuclear membranes break down where they are in contact with each other (Fig. 8). At the same time the chromatin of each condenses into a fine spireme. The nuclear sap of the female pronucleus simultaneously develops an affinity for light green, so that the two pronuclei can no longer be distinguished. The spiremes of the two nuclei become coarser and intermingle before any distinct chromosomes are visible (Fig. 11). The maturation divisions occupy about one and one-half hours each. Union of the pronuclei occurs during the fourth hour after oviposition.

### *Affinities of Lepismatids*

The lepismatids for which both embryological and morphological data are available are *Lepisma saccharina* L. (Heymons, 1896, 1897; Uzel, 1897, 1898), *Thermobia domestica* (Packard) (Woodland, 1952, 1957; Sahrhage, 1953; Wellhouse, 1953), and *Ctenolepisma lineata* Fabricius (Woodland, 1957). All three belong to Subfamily Lepismatinae. The data indicate that *Thermobia domestica* and *Ctenolepisma lineata* are very closely related to each other and less closely related to *Lepisma saccharina,* which is more primitive than they.

*Thermobia domestica* and *Ctenolepisma lineata* differ embryologically from *Lepisma saccharina* in that in them the germ disk is formed at the extreme posterior end of the egg, radial symmetry is maintained until the germ disk starts to elongate, the embryo does not sink bodily into the yolk, the amniotic pore is not closed by a chitinous plug, and spiracle anlagen are not found on the ninth and tenth abdominal segments. Adults of *Thermobia domestica* and *Ctenolepisma lineata* differ from those of *Lepisma saccharina* by lacking parameres and by possessing ventricular caecae, an anterior projection of the vas deferens beyond where the most anterior pair of vasa efferentia empties into it, and often a third pair of styli. In the former two species the vasa efferentia of the members of a pair of sperm tubes do not unite before joining the vas deferens and the free end of one member of each pair of sperm tubes is directed mediad, while the free end of the other member is directed laterad. In addition, the lateral oviducts are relatively

shorter than in *Lepisma saccharina.* The author considers all of these characters to be secondary or specialized ones. Remington (personal communication, 1949) has noted several taxonomic characters as evidence for considering *Lepisma saccharina* more primitive than the other two species: the cephalic hairs of the former occur singly and do not bear secondary hairs, while those of the latter occur in groups and bear secondary hairs; also, the former has a less well developed ovipositor.

In spite of the close relationship between *Thermobia domestica* and *Ctenolepisma lineata,* each has a few specialized characters not shared by the other. In *Ctenolepisma lineata* a modified morula stage is absent, synchrony of cleavage is maintained longer, and intravitelline separation occurs late. In *Thermobia domestica* the amnio-serosal folds develop only weakly and there is a transverse division of the distal segment of the maxillary palpus.

Although the embryology of lepismatids and symphylans appears superficially very different, owing to the difference in relative amounts of yolk in the eggs of the two groups, actually a great deal of embryological affinity exists between them. Some of the characters shared by *Thermobia domestica* and the symphylan *Hanseniella agilis* (Tiegs, 1940) are of particular interest: (1) The polar bodies are not extruded from the egg and degenerate rapidly. (2) A vitelline membrane is not apparent. (3) Periplasm has not been identified in freshly laid eggs. (4) The inner layer is entirely mesodermal and is produced without the formation of a ventral groove. (5) The time and manner of segregation of the germ cells are similar. (6) The surface cells secrete a cuticle, in *Hanseniella* before germ band formation, in *Thermobia* after germ disk formation. (7) The midgut epithelium is derived from yolk cells. (8) The neuropile of the nerve cord is not covered dorsally by nerve cells. (9) Fourteen post-cephalic segments occur in the adult. Anamorphosis occurs in *Hanseniella* but not, of course, in *Thermobia.* It may be noted, however, that *Hanseniella* hatches with 8 post-cephalic segments followed by a pre-anal and an anal segment. During anamorphosis 4 more segments differentiate in front of the pre-anal seg-

ment. In *Thermobia* a distinct pause occurs after differentiation of the first 5 abdominal (first 8 post-cephalic) segments. The cercus-bearing (pre-anal) and anal segments are prominent during the interval before the remaining abdominal segments are differentiated in front of them.

The characters in which *Hanseniella* differs from *Thermobia* may be grouped into several categories. Examples of characters with homologs in some primitive or generalized insects, but not in *Thermobia,* are: (1) Paired arteries arise from the aorta. (2) The presence of eversible sacs and coxal styli. Homologs of both of these structures are present in *Machilis.* In *Hanseniella* the two halves of the nerve cord arise laterally from the floor of the germ band rather than medially as in *Thermobia.* In *Hanseniella* the medial position is occupied by the "ventral organs" from which the eversible sacs arise. Examples of characters more primitive than those found in primitive insects are: (1) The adult has abdominal legs. (2) Only one pair of Malpighian tubules is present. (3) The labial segment is at first not part of the head. A few of the characters of *Hanseniella,* such as the secondarily acquired progoneate condition and the development of fat body from yolk cells, seem to express affinity with diplopods or as the incorporation of a pre-antennary ganglion into the brain, with chilopods. Other characters of *Hanseniella,* as the absence of eyes and the presence of only a single pair of tracheae (cephalic), are specializations which are probably adaptations to its environment of decaying foliage and rotting logs.

The following embryological differences between *Hanseniella* and lepismatids are considered by the author to be the result largely of the difference in relative volume of yolk in their eggs. In *Hanseniella*: (1) Cleavage is total. (2) The germ band is long and from the beginning represents thoracic and abdominal as well as cephalic material. (3) Absence of embryonic membranes; the germ band does not sink into the yolk. (4) Early eclosion, with subsequent anamorphosis. Actually nutritive value of the yolk rather than volume is concerned here. Information is not available on this subject in insects.

Tiegs (1940), however, deems it erroneous to consider the type of cleavage as simply the mechanical result of the quantity of yolk within the egg. In support of this contention he says that the eggs of symphylans are not unusually small, though cleavage is total. But consider the following comparison. The egg of *Hanseniella* is spherical, averaging 0.37 mm. in diameter; the long diameter of the ellipsoidal egg of *Thermobia* averages 1.00 mm., the short diameter 0.80 mm. At hatching, *Thermobia* measures 1.5 mm. long, exclusive of appendages. We deduce that *Hanseniella* also measures close to 1.5 mm. long at hatching, for the following reason. The circumference of a sphere 0.37 mm. in diameter is 1.2 mm. The body of the embryo is curved in a circle around the entire circumference of the egg, but is a little longer than the circumference since the bent head is directed inward. We therefore conclude that the egg of *Thermobia* contains a relatively much larger percentage of yolk than does the egg of *Hanseniella*.

Tiegs further supports his statement by noting that some tiny, yolkless insect eggs have superficial cleavage (Fernando, 1934). But there is no reason to suppose that superficial cleavage, once established, would not be as satisfactory for yolkless as for yolk-rich eggs. We do not consider, however, that this fact invalidates the theory that superficial cleavage was originally developed as an adaptation to large, yolk-rich eggs.

Although only one species of perlarian has been thoroughly studied embryologically, Miller's detailed account (1939, 1940) of *Pteronarcys proteus* reveals a number of similarities between the embryology of this perlarian and that of lepismatids: (1) Practically no cytoplasm is present in freshly laid eggs. (2) The embryonic rudiment represents chiefly cephalic material. (3) The germ band is of the immersed type. (4) The inner layer represents mesoderm only and is produced without the formation of a ventral furrow. (5) The mesoderm of the eleventh abdominal segment shows no coelomic sacs. (6) Transitory appendages appear on the intercalary segment and on abdominal segments two to ten.

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